MAD Data Collection on a Samarium Derivative of InlB, a Surface-attached Protein of the Bacterial Pathogen *Listeria monocytogenes*

M. Marino and P. Ghosh (U. of California, San Diego)

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Introduction: InlB is a 67 kD surface-attached protein of the bacterial pathogen *Listeria monocytogenes* that promotes bacterial invasion of diverse mammalian cell types. The protein triggers an intracellular signaling cascade involving activation of phosphoinositide 3-kinase. The structure of the leucine-rich repeats domain of InlB demonstrated the presence of two unsuspected calcium-binding sites (Marino *et al*., 1999). Based on the presence of these sites, we designed a multiwavelength anomalous dispersion (MAD) experiment utilizing samarium, a lanthanide that has been shown to substitute for calcium, to determine the X-ray crystal structure of intact InlB.

Methods and Materials: Crystals of intact InlB were derivatized with $SmCl_3$, soaked in an erythritol-containing cryobuffer, and flash-cooled at ~100 K. Several MAD data sets utilizing three wavelengths (peak, inflection, and remote) corresponding to the samarium L_{III} edge were collected at X4A.

Results: Analysis of the MAD experiment using SOLVE indicated that both anomalous and dispersive difference signals yielded useful phasing information. Unexpectedly crystals of InlB suffered radiation damage during the course of data collection. With this knowledge in hand, future MAD experiments on crystals of intact InlB will be optimized for speed of data collection. Phasing information appears to extend to ~3.5 Å, and given the high solvent content of the crystals of 75% should yield a structural determination with the aid of solvent flattening and phase extension. Initial electron density maps have been calculated and model building is in progress.

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References: M. Marino, L. Braun, P. Cossart, and P. Ghosh “Structure of the InlB leucine-rich repeats, a domain that triggers host cell invasion by the bacterial pathogen *L. monocytogenes,*” Molecular Cell 4, 1063-1072, 1999.